

Supporting Material for “Stochastic model of clathrin-coated pit assembly”

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1 Values of parameters appearing in Eq. 1 of main text

The value of the membrane bending rigidity κ_m is typically between $10 kT$ and $25 kT$ [1]. In our analysis we keep the value fixed at $20 kT$. We choose the spontaneous curvature of the protein coat, c_p , to be $1/45$ nm. The reasoning behind this choice is that the most abundant proteins in the coat, clathrin and its adapter AP-2, are known to form clathrin baskets of similar curvature [2]. We choose the curvature of a pit, $\bar{c} = 1/R = 1/50$ nm, and assume that it does not change as the pit grows in size. This assumption is satisfied if $\sigma \ll \kappa_p$, in which case the curvature can be written as (see Eq. 3 in the main text)

$$\bar{c} = \frac{\kappa_p c_p}{\kappa_p + \kappa_m} = \frac{1}{50}. \quad (\text{S1})$$

Substituting the values of κ_m and c_p in the above equation, we get $\kappa_p = 200 kT$. This value is comparable to, but less than, the bending modulus of a CCV (approximately $285 kT$) determined by atomic force microscopy [3]. The effective binding energy b is a free parameter in our analysis. We determine its value by fitting experimental data on lifetime distribution of CCPs. However, to get a lower bound estimate for b we use the idea that in a CCV the total binding energy must be comparable to the total bending energy. The energy required to bend a flat cell membrane into a spherical vesicle is $8\pi\kappa_m \approx 500 kT$ [4]. In our model, a vesicle has 100 monomers, so the effective binding energy b has to be at least $500 kT/100 = 5 kT$. Thus, in our analysis we search for b value in the vicinity of $5 kT$. The edge energy constant σ is also a free parameter in our analysis. Since the edge energy is a correction to the binding energy, the value of σ should be less than b . In our simulations, we search for σ value in the vicinity of $1 kT$. A CCV of radius $R = 50$ nm contains approximately $N = 100$ clathrin molecules [5]. Thus the average area occupied by a monomer, $\lambda = 4\pi R^2/N = 314 \text{ nm}^2$. In our analysis we choose $\lambda = 310 \text{ nm}^2$.

2 Monte Carlo simulations of lifetime distribution of abortive pits

During TIRF measurements, when a CCP first becomes visible under the microscope, it already has assembled into a structure containing several clathrin molecules. To make our simulations commensurate with the experiments, we assume that at $t = 0$, a pit has 5 monomers. We also assume that if the number of monomers on a pit falls to 4, such occurrence results in termination of the pit.

The lifetime distribution of abortive pits were obtained through kinetic Monte Carlo simulation [6]. The simulation algorithm is the following:

1. Start with pit of size $n = 5$. Set time $t = 0$.

2. Form a list of all the rates of possible transition. In our case, a pit of size n can grow to size $n + 1$ or decay to size $n - 1$ with rate constants α_n and β_n respectively.
3. Calculate the cumulative rate $K = \alpha_n + \beta_n$.
4. Generate a random number ξ_1 , uniformly distributed between 0 and 1.
5. If $\xi_1 > \beta_n/K$, increase CCP size by 1, otherwise decrease it by 1.
6. Increment the time as $t = t - \log(\xi_2)/K$, where ξ_2 is also a random number uniformly distributed between 0 and 1.
7. Return to step to step 1 until $n = 4$ or $n = N = 100$ (vesicle size). When $n = 4$ (abortive fate) or 100 (productive fate), stop the simulation and record the fate, and time t as the lifetime of the pit.

3 Number of available binding sites on the perimeter of a pit

Consider a pit (partially formed sphere) of radius $R = 1/\bar{c}$, where \bar{c} is the curvature (see Fig.S1). Let λ be the average area occupied by a monomer in a pit. Let r be the radius of the circular periphery of the pit. Using spherical coordinates (r, θ, ϕ) , the surface area of the pit can be written as

$$A(\theta) = 2\pi r^2[1 - \cos(\theta)] = \lambda n. \quad (\text{S2})$$

This leads to

$$\cos(\theta) = 1 - \frac{A}{2\pi R^2} = 1 - \frac{\lambda n \bar{c}^2}{2\pi}. \quad (\text{S3})$$

From the above equation, we get the radius of the circular periphery to be

$$r(n) = R \sin(\theta) = R \sqrt{[1 + \cos(\theta)][1 - \cos(\theta)]} = \sqrt{\frac{\lambda n}{\pi}} \times \sqrt{1 - \frac{\lambda n \bar{c}^2}{4\pi}}. \quad (\text{S4})$$

Thus, the number of available binding sites on the periphery of the pit is

$$f(n) = \frac{2\pi r(n)}{d} = \frac{2\pi}{d} \sqrt{\frac{\lambda n}{\pi}} \sqrt{1 - \frac{\lambda n \bar{c}^2}{4\pi}}, \quad (\text{S5})$$

where d is the average span of a monomer. We use Eq.S5 in the main text.

For a vesicle containing 100 clathrin molecules, the enumeration of clathrin shows that their number on the edge, when the vesicle is half complete (hemisphere), is approximately 10. We find that for $d = 1.8\sqrt{\lambda}$ the number of available binding sites when pit is close to a hemisphere is around 10 (see Fig. S2). Therefore, we choose $d = 1.8\sqrt{\lambda}$.

4 Lifetime distribution of CCPs in Ref. [7]

Fig.1D in Ref. [7] contains the plot of the lifetime distribution of CCPs. The plot includes the lifetimes of both abortive and productive CCPs. Since we are interested in the lifetime distribution of abortive CCPs, we make the assumption that all the data points with lifetimes less than 30 sec correspond to the abortive CCPs. In addition, in Ref. [7] to get rid of the transient, highly motile structures CCPs with lifetime less than 2 sec were not considered. Thus, while calculating the lifetime distribution of abortive pits, shown in Fig. 3 of the main text, we consider only the abortive pits with lifetime greater than 2 sec.

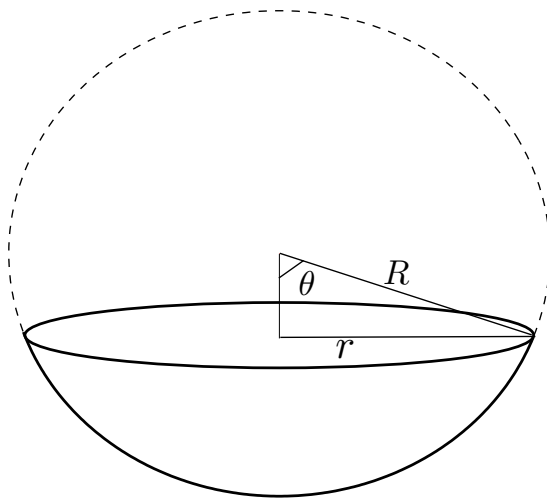


Figure S1: Spherical cap representing the assumed shape of a pit in our model.

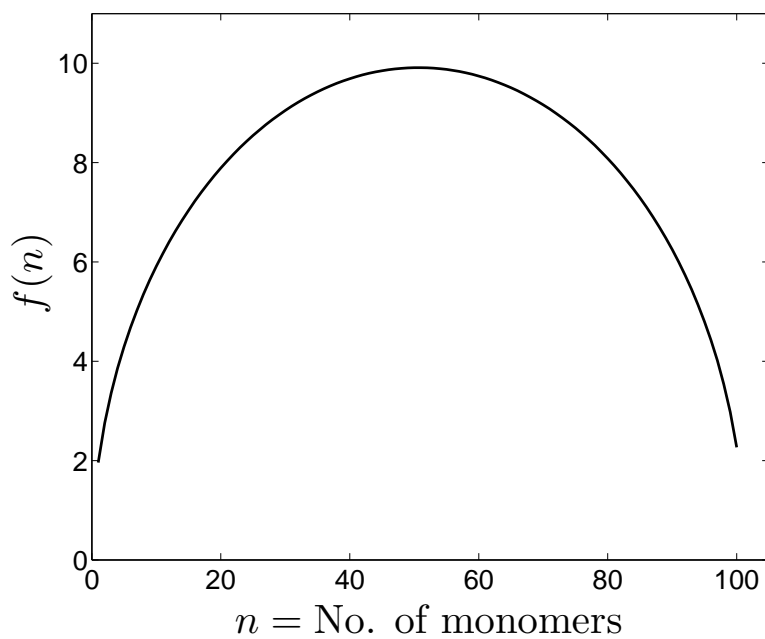


Figure S2: Available binding sites on the periphery of a pit of radius 50 nm, as a function of pit size, n .

5 Detailed balance

The condition of detailed balance implies that, at equilibrium, the net flux between sites n and $n + 1$ must be zero, i.e.,

$$\alpha_n P_n^{eq} = \beta_{n+1} P_{n+1}^{eq}, \quad (S6)$$

where P_n^{eq} is the equilibrium distribution. At equilibrium, $P_n^{eq} \sim \exp[-F(n)]$, where $F(n) = E(n) - nkT \ln(mv_0)$ is the free energy of a pit of size n , m is the concentration of free monomers and v_0 is a quantity that has units of volume. The backward rate constants then can be written as

$$\beta_{n+1} = \alpha_n \frac{P_n^{eq}}{P_{n+1}^{eq}} = \alpha_n \exp[F(n+1) - F(n)]. \quad (S7)$$

Substituting the formula for the forward rate constant used in the main text, i.e., $\alpha_n = k_b m f(n)$, $n = 1, 2, \dots, N - 1$, in the above equation we get

$$\beta_{n+1} = \frac{k_b}{v_0} f(n) \exp[E(n+1) - E(n)] = \mu f(n) \exp[E(n+1) - E(n)], \quad (S8)$$

where $\mu = k_b/v_0$. In the main text we use the form of backward rate constant given in Eq. S8.

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